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(54) Title: KERATINOCYTE GROWTH FACTOR-2 (57) Abstract A human polypeptide and DNA (RNA) encoding such polypeptide and procedure for producing such polypeptide by recombinant techniques is disclosed. Also disclosed are methods for utilizing such polypeptide for stimulating epithelial cell growth which may be used to stimulate wound healing, reduce scarring and prevent hair loss. Antagonists against such polypeptides and their use as a therapeutic to treat proliferative diseases such as cancer, psoriasis, Kaposi's sarcoma, keloids, retinopathy and restenosis are also disclosed. Diagnostic methods for detecting mutations in the KGF-2 coding sequence and alterations in the concentration of KGF-2 protein in a sample derived from a host are also disclosed.		

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KERATINOCYTE GROWTH FACTOR-2

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptide of the present invention is a Keratinocyte growth factor, sometimes hereinafter referred to as "KGF-2". The invention also relates to inhibiting the action of such polypeptides.

The fibroblast growth factor family has emerged as a large family of growth factors involved in soft-tissue growth and regeneration. It presently includes several members that share a varying degree of homology at the protein level, and that, with one exception, appear to have a similar broad mitogenic spectrum, i.e., they promote the proliferation of a variety of cells of mesodermal and neuroectodermal origin and/or promote angiogenesis.

The pattern of expression of the different members of the family is very different, ranging from extremely restricted expressions of some stages of development, to rather ubiquitous expression in a variety of tissues and organs. All the members appear to bind heparin and heparin sulfate proteoglycans and glycosaminoglycans and strongly

concentrate in the extracellular matrix. KGF was originally identified as a member of the FGF family by sequence homology or factor purification and cloning.

Keratinocyte growth factor (KGF) was isolated as a mitogen for a cultured murine keratinocyte line (Rubin, J.S., et al., PNAS, USA, 86:802-806 (1989)). Unlike the other members of the FGF family, it has little activity on mesenchyme-derived cells but stimulates the growth of epithelial cells. The Keratinocyte growth factor gene encodes a 194-amino acid polypeptide (Finch, P.W., et al., Science, 245:752-755 (1989)). The N-terminal 64 amino acids are unique, but the remainder of the protein has about 30% homology to bFGF. KGF is the most divergent member of the FGF family. The molecule has a hydrophobic signal sequence and is efficiently secreted. Post-translational modifications include cleavage of the signal sequence and N-linked glycosylation at one site, resulting in a protein of 28 kDa. Keratinocyte growth factor is produced by fibroblast derived from skin and fetal lung, (Rubin, et al., (1989)). The Keratinocyte growth factor mRNA was found to be expressed in adult kidney, colon and ilium, but not in brain or lung (Finch, P.W., et al., Science, 245:752-755 (1989)). KGF displays the conserved regions within the FGF protein family. KGF binds to the FGF-2 receptor with high affinity.

The polypeptide of the present invention has been putatively identified as a member of the FGF family, more particularly the polypeptide has been putatively identified as KGF-2 as a result of amino acid sequence homology with other members of the FGF family.

In accordance with one aspect of the present invention, there are provided novel mature polypeptides which are KGF-2 as well as biologically active and diagnostically or therapeutically useful fragments, analogs and derivatives thereof. The polypeptides of the present invention are of human origin.

In accordance with one aspect of the present invention, there are provided isolated nucleic acid molecules encoding human KGF-2, including mRNAs, DNAs, cDNAs, genomic DNA, as well as antisense analogs thereof and biologically active and diagnostically or therapeutically useful fragments thereof.

In accordance with another aspect of the present invention, there is provided a process for producing such polypeptide by recombinant techniques through the use of recombinant vectors, such as cloning and expression plasmids useful as reagents in the recombinant production of KGF-2 proteins, as well as recombinant prokaryotic and/or eukaryotic host cells comprising a human KGF-2 nucleic acid sequence.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptide, or polynucleotide encoding such polypeptide for therapeutic purposes, for example, to stimulate epithelial cell proliferation for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds.

In accordance with yet a further aspect of the present invention, there are provided antibodies against such polypeptides.

In accordance with another aspect of the present invention, there are provided nucleic acid probes comprising nucleic acid molecules of sufficient length to specifically hybridize to human KGF-2 sequences.

In accordance with yet another aspect of the present invention, there are provided antagonists to such polypeptides, which may be used to inhibit the action of such polypeptides, for example, to reduce scarring during the wound healing process and to prevent and/or treat tumor proliferation, diabetic retinopathy, rheumatoid arthritis and tumor growth.

In accordance with yet another aspect of the present invention, there are provided diagnostic assays for detecting diseases or susceptibility to diseases related to mutations in KGF-2 nucleic acid sequences or over-expression of the polypeptides encoded by such sequences.

In accordance with another aspect of the present invention, there is provided a process for utilizing such polypeptides, or polynucleotides encoding such polypeptides, for *in vitro* purposes related to scientific research, synthesis of DNA and manufacture of DNA vectors.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figure 1 illustrates the cDNA and corresponding deduced amino acid sequence of the polypeptide of the present invention. The initial 36 amino acid residues represent the putative leader sequence (underlined). The standard one-letter abbreviations for amino acids are used. Sequencing inaccuracies are a common problem when attempting to determine polynucleotide sequences. Sequencing was performed using a 373 Automated DNA sequencer (Applied Biosystems, Inc.). Sequencing accuracy is predicted to be greater than 97% accurate.

Figure 2 is an illustration of a comparison of the amino acid sequence of the polypeptide of the present invention and other fibroblast growth factors.

In accordance with an aspect of the present invention, there is provided an isolated nucleic acid (polynucleotide) which encodes for the mature polypeptide having the deduced amino acid sequence of Figure 1 (SEQ ID No. 2) or for the mature polypeptide encoded by the cDNA of the clone deposited as ATCC Deposit No. 75977 on December 16, 1994.

A polynucleotide encoding a polypeptide of the present invention may be obtained from a human prostate and fetal lung. A fragment of the cDNA encoding the polypeptide was initially isolated from a library derived from a human normal prostate. The open reading frame encoding the full length protein was subsequently isolated from a randomly primed human fetal lung cDNA library. It is structurally related to the FGF family. It contains an open reading frame encoding a protein of 208 amino acid residues of which approximately the first 36 amino acid residues are the putative leader sequence such that the mature protein comprises 172 amino acids. The protein exhibits the highest degree of homology to human keratinocyte growth factor with 45 % identity and 82 % similarity over a 206 amino acid stretch. It is also important that sequences that are conserved through the FGF family are found to be conserved in the protein of the present invention.

The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptide may be identical to the coding sequence shown in Figure 1 (SEQ ID No. 1) or that of the deposited clone or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature polypeptide as the DNA of Figure 1 (SEQ ID No. 1) or the deposited cDNA.

The polynucleotide which encodes for the mature polypeptide of Figure 1 (SEQ ID No. 2) or for the mature polypeptide encoded by the deposited cDNA may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence such as a leader or secretory sequence or a

proprotein sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of Figure 1 (SEQ ID No. 2) or the polypeptide encoded by the cDNA of the deposited clone. The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same mature polypeptide as shown in Figure 1 (SEQ ID No. 2) or the same mature polypeptide encoded by the cDNA of the deposited clone as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptide of Figure 1 (SEQ ID No. 2) or the polypeptide encoded by the cDNA of the deposited clone. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotide may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in Figure 1 (SEQ ID No. 1) or of the coding sequence of the deposited clone. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does

not substantially alter the function of the encoded polypeptide.

The present invention also includes polynucleotides, wherein the coding sequence for the mature polypeptide may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the mature form of the polypeptide. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.

Thus, for example, the polynucleotide of the present invention may encode for a mature protein, or for a protein having a prosequence or for a protein having both a prosequence and a presequence (leader sequence).

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexahistidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 50% and preferably 70% identity between the sequences. The present invention

particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95 % and preferably at least 97 % identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNA of Figure 1 (SEQ ID No. 1) or the deposited cDNA.

The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

The present invention further relates to a polypeptide which has the deduced amino acid sequence of Figure 1 (SEQ ID No. 2) or which has the amino acid sequence encoded by the deposited cDNA, as well as fragments, analogs and derivatives of such polypeptide.

The terms "fragment," "derivative" and "analog" when referring to the polypeptide of Figure 1 (SEQ ID No. 2) or that encoded by the deposited cDNA, means a polypeptide which retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a proprotein

which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The fragment, derivative or analog of the polypeptide of Figure 1 (SEQ ID No. 2) or that encoded by the deposited cDNA may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a

composition, and still be isolated in that such vector or composition is not part of its natural environment.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the KGF-2 genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing polypeptides by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such

procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spodoptera Sf9; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into

which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example; Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pBS, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α -factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences,

and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well known to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The KGF-2 polypeptide can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques

from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

The polypeptide of the present invention may be employed to stimulate new blood vessel growth or angiogenesis. Particularly, the polypeptide of the present invention may stimulate keratinocyte cell growth and proliferation. Accordingly, the polypeptide of the present invention may be used to stimulate wound healing, and also to stimulate Keratinocytes which is related to the prevention of hair loss.

The polypeptide of the present invention may also be employed to heal dermal wounds by stimulating epithelial cell proliferation.

The polypeptide of the present invention may also be employed to stimulate differentiation of cells, for example, muscle cells and nervous tissue, prostate cells and lung cells.

The signal sequence of KGF-2 encoding amino acids 1 through 36 may be employed to identify secreted proteins in general by hybridization and/or computational search algorithms.

The nucleotide sequence of KGF-2 could be employed to isolate 5' sequences by hybridization. Plasmids comprising the KGF-2 gene under the control of its native promoter/enhancer sequences could then be used in in vitro studies aimed at the identification of endogenous cellular and viral transactivators of KGF-2 gene expression.

The KGF-2 protein may also be employed as a positive control in experiments designed to identify peptido-mimetics acting upon the KGF-2 receptor.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptides, or polynucleotides encoding such polypeptides, for in vitro purposes related to scientific research, synthesis of DNA, manufacture of DNA vectors and for the purpose of providing diagnostics and therapeutics for the treatment of human disease.

Fragments of the full length KGF-2 gene may be used as a hybridization probe for a cDNA library to isolate the full length KGF-2 genes and to isolate other genes which have a high sequence similarity to these genes or similar biological activity. Probes of this type generally have at least 20 bases. Preferably, however, the probes have at least 30 bases and generally do not exceed 50 bases, although they may have a greater number of bases. The probe may also be used to identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete KGF-2 gene including regulatory and promotor regions, exons, and introns. An example of a screen comprises isolating the coding region of the KGF-2 gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to that of the gene of the present invention are used to screen a library of human cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

This invention provides a method for identification of the receptors for the KGF-2 polypeptide. The gene encoding the receptor can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, and a cDNA library created from this RNA is divided into pools and used to transfect COS

cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the labeled polypeptides. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to x-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

This invention provides a method of screening compounds to identify those which agonize the action of KGF-2 or block the function of KGF-2. An example of such an assay comprises combining a mammalian Keratinocyte cell, the compound to be screened and ³[H] thymidine under cell culture conditions where the keratinocyte cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of keratinocyte proliferation in the presence of the compound to determine if the compound stimulates proliferation of Keratinocytes.

To screen for antagonists, the same assay may be prepared in the presence of KGF-2 and the ability of the compound to prevent Keratinocyte proliferation is measured

and a determination of antagonist ability is made. The amount of Keratinocyte cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of $^3\text{[H]}$ thymidine.

In another method, a mammalian cell or membrane preparation expressing the KGF-2 receptor would be incubated with labeled KGF-2 in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of KGF-2 and receptor would be measured and compared in the presence or absence of the compound. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

Examples of potential KGF-2 antagonists include an antibody, or in some cases, an oligonucleotide, which binds to the polypeptide. Alternatively, a potential KGF-2 antagonist may be a mutant form of KGF-2 which binds to KGF-2 receptors, however, no second messenger response is elicited and therefore the action of KGF-2 is effectively blocked.

Another potential KGF-2 antagonist is an antisense construct prepared using antisense technology. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the mature polypeptides of the present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix -see Lee et al., Nucl. Acids Res., 6:3073 (1979); Cooney et al, Science, 241:456 (1988); and Dervan et al., Science, 251: 1360 (1991)), thereby preventing transcription and the production of KGF-2. The

antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into KGF-2 polypeptide (Antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of KGF-2.

Potential KGF-2 antagonists include small molecules which bind to and occupy the binding site of the KGF-2 receptor thereby making the receptor inaccessible to KGF-2 such that normal biological activity is prevented. Examples of small molecules include but are not limited to small peptides or peptide-like molecules.

The KGF-2 antagonists may be employed to prevent the induction of new blood vessel growth or angiogenesis in tumors. Angiogenesis stimulated by KGF-2 also contributes to several pathologies which may also be treated by the antagonists of the present invention, including diabetic retinopathy, and inhibition of the growth of pathological tissues, such as in rheumatoid arthritis.

KGF-2 antagonists may also be employed to treat glomerulonephritis, which is characterized by the marked proliferation of glomerular epithelial cells which form a cellular mass filling Bowman's space.

The antagonists may also be employed to inhibit the over-production of scar tissue seen in keloid formation after surgery, fibrosis after myocardial infarction or fibrotic lesions associated with pulmonary fibrosis and restenosis. KGF-2 antagonists may also be employed to treat other proliferative diseases which are stimulated by KGF-2, including cancer and Kaposi's sarcoma.

KGF-2 antagonists may also be employed to treat keratitis which is a chronic infiltration of the deep layers

of the cornea with uveal inflammation characterized by epithelial cell proliferation.

The antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

The polypeptides, agonists and antagonists of the present invention may be employed in combination with a suitable pharmaceutical carrier to comprise a pharmaceutical composition. Such compositions comprise a therapeutically effective amount of the polypeptide, agonist or antagonist and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides, agonists and antagonists of the present invention may be employed in conjunction with other therapeutic compounds.

The pharmaceutical compositions may be administered in a convenient manner such as by the oral, topical, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. The pharmaceutical compositions are administered in an amount which is effective for treating and/or prophylaxis of the specific indication. In general, they are administered in an amount of at least about 10 $\mu\text{g/kg}$ body weight and in most cases they will be administered in an amount not in excess of about 8 mg/Kg body

weight per day. In most cases, the dosage is from about 10 $\mu\text{g/kg}$ to about 1 mg/kg body weight daily, taking into account the routes of administration, symptoms, etc. In the specific case of topical administration dosages are preferably administered from about 0.1 μg to 9 mg per cm^2 .

The KGF-2 polypeptides, agonists and antagonists which are polypeptides may also be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by, for example, procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells *in vivo* after combination with a suitable delivery vehicle. Examples of other delivery vehicles include an HSV-based vector system, adeno-associated virus vectors, and inert vehicles, for example, dextran coated ferrite particles.

This invention is also related to the use of the KGF-2 gene as part of a diagnostic assay for detecting diseases or susceptibility to diseases related to the presence of mutations in the KGF-2 nucleic acid sequences.

Individuals carrying mutations in the KGF-2 gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki et al., *Nature*, 324:163-166 (1986)) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid encoding KGF-2 can be used to identify and analyze KGF-2 mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled KGF-2 RNA or alternatively, radiolabeled KGF-2 antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers et al., *Science*, 230:1242 (1985)).

Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1

protection or the chemical cleavage method (e.g., Cotton et al., PNAS, USA, 85:4397-4401 (1985)).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., Restriction Fragment Length Polymorphisms (RFLP)) and Southern blotting of genomic DNA.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

The present invention also relates to a diagnostic assay for detecting altered levels of KGF-2 protein in various tissues since an over-expression of the proteins compared to normal control tissue samples may detect the presence of a disease or susceptibility to a disease, for example, a tumor. Assays used to detect levels of KGF-2 protein in a sample derived from a host are well-known to those of skill in the art and include radioimmunoassays, competitive-binding assays, Western Blot analysis, ELISA assays and "sandwich" assay. An ELISA assay (Coligan, et al., Current Protocols in Immunology, 1(2), Chapter 6, (1991)) initially comprises preparing an antibody specific to the KGF-2 antigen, preferably a monoclonal antibody. In addition a reporter antibody is prepared against the monoclonal antibody. To the reporter antibody is attached a detectable reagent such as radioactivity, fluorescence or, in this example, a horseradish peroxidase enzyme. A sample is removed from a host and incubated on a solid support, e.g. a polystyrene dish, that binds the proteins in the sample. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein like bovine serum albumen. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any KGF-2 proteins attached to the polystyrene dish. All unbound monoclonal antibody is washed out with buffer. The

reporter antibody linked to horseradish peroxidase is now placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to KGF-2. Unattached reporter antibody is then washed out. Peroxidase substrates are then added to the dish and the amount of color developed in a given time period is a measurement of the amount of KGF-2 protein present in a given volume of patient sample when compared against a standard curve.

A competition assay may be employed wherein antibodies specific to KGF-2 are attached to a solid support and labeled KGF-2 and a sample derived from the host are passed over the solid support and the amount of label detected, for example by liquid scintillation chromatography, can be correlated to a quantity of KGF-2 in the sample.

A "sandwich" assay is similar to an ELISA assay. In a "sandwich" assay KGF-2 is passed over a solid support and binds to antibody attached to a solid support. A second antibody is then bound to the KGF-2. A third antibody which is labeled and specific to the second antibody is then passed over the solid support and binds to the second antibody and an amount can then be quantified.

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region is used to

rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 500 or 600 bases; however, clones larger than 2,000 bp have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. FISH requires use of the clones from which the EST was derived, and the longer the better. For example, 2,000 bp is good, 4,000 is better, and more than 4,000 is probably not necessary to get good results a reasonable percentage of the time. For a review of this technique, see Verma et al., *Human Chromosomes: a Manual of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man* (available on line through Johns Hopkins University Welch Medical Library). The relationship between

genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction

conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. et al., *Nucleic Acids Res.*, 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., *Id.*, p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units of T4 DNA ligase ("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently

linked) inter-chromosomal DNA making the genome of the cell. Prokaryote and yeast, for example, the exogenous DNA may be maintained on an episomal element, such a plasmid. With respect to eukaryotic cells, a stably transformed or transfected cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This ability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cell containing the exogenous DNA. An example of transformation is exhibited in Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

"Transduction" or "transduced" refers to a process by which cells take up foreign DNA and integrate that foreign DNA into their chromosome. Transduction can be accomplished, for example, by transfection, which refers to various techniques by which cells take up DNA, or infection, by which viruses are used to transfer DNA into cells.

Example 1

Bacterial Expression and Purification of KGF-2

The DNA sequence encoding KGF-2, ATCC # 75977, is initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' end sequences of the processed KGF-2 cDNA (including the signal peptide sequence). The 5' oligonucleotide primer has the sequence 5' CCCCACATGTGGAAATGGATACTGACACATTGTGCC 3' (SEQ ID No. 3) contains an Afl III restriction enzyme site including and followed by 30 nucleotides of KGF-2 coding sequence starting from the presumed initiation codon. The 3' sequence 5' CCCAAGCTTCCACAAACGTTGCCTTCCTCTATGAG 3' (SEQ ID No. 4) contains complementary sequences to Hind III site and is followed by 26 nucleotides of KGF-2. The restriction enzyme sites are compatible with the restriction enzyme sites on the bacterial expression vector pQE-60 (Qiagen, Inc. Chatsworth,

CA). pQE-60 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-60 is then digested with NcoI and HindIII. The amplified sequences are ligated into pQE-60 and are inserted in frame. The ligation mixture is then used to transform E. coli strain M15/rep 4 (Qiagen, Inc.) by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized KGF-2 is purified from this solution by chromatography on a Heparin affinity column under conditions that allow for tight binding of the proteins (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). KGF-2 (75 % pure) is eluted from the column by high salt buffer.

Example 2Bacterial Expression and Purification of a truncated version of KGF-2

The DNA sequence encoding KGF-2, ATCC # 75977, is initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the truncated version of the KGF-2 polypeptide. The truncated version comprises the polypeptide minus the 36 amino acid signal sequence, with a methionine and alanine residue being added just before the cysteine residue which comprises amino acid 37 of the full-length protein. The 5' oligonucleotide primer has the sequence 5' CATGCCATGGCGTGCCAAGCCCTTGGTCAGGACATG 3' (SEQ ID No. 5) contains an NcoI restriction enzyme site including and followed by 24 nucleotides of KGF-2 coding sequence. The 3' sequence 5' CCCAAGCTTCCACAAACGTTGCCTTCCTC TATGAG 3' (SEQ ID No. 6) contains complementary sequences to Hind III site and is followed by 26 nucleotides of the KGF-2 gene. The restriction enzyme sites are compatible with the restriction enzyme sites on the bacterial expression vector pQE-60 (Qiagen, Inc. Chatsworth, CA). pQE-60 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-60 is then digested with NcoI and HindIII. The amplified sequences are ligated into pQE-60 and are inserted in frame. The ligation mixture is then used to transform E. coli strain M15/rep 4 (Qiagen, Inc.) by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis. Clones containing the

desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized KGF-2 is purified from this solution by chromatography on a Heparin affinity column under conditions that allow for tight binding the proteins (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). KGF-2 protein is eluted from the column by high salt buffer.

Example 3

Cloning and expression of KGF-2 using the baculovirus expression system

The DNA sequence encoding the full length KGF-2 protein, ATCC # 75977, is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

The 5' primer has the sequence 5' GCGGGATCCGCCATC**ATGT**GGAATGGATACTCAC 3' (SEQ ID No. 7) and contains a BamHI restriction enzyme site (in bold) followed by 6 nucleotides resembling an efficient signal for the initiation of translation in eukaryotic cells (Kozak, M., J. Mol. Biol., 196:947-950 (1987)). and just behind the first 17 nucleotides of the KGF-2 gene (the initiation codon for translation "ATG" is underlined).

The 3' primer has the sequence 5' GCGCGGTACCAAAACGTTGCCTTCCT 3' (SEQ ID No. 8) and contains the cleavage site for the restriction endonuclease Asp718 and

19 nucleotides complementary to the 3' non-translated sequence of the KGF-2 gene. The amplified sequences are isolated from a 1% agarose gel using a commercially available kit from Qiagen, Inc., Chatsworth, CA. The fragment is then digested with the endonucleases BamHI and Asp718 and then purified again on a 1% agarose gel. This fragment is designated F2.

The vector pA2 (modification of pVL941 vector, discussed below) is used for the expression of the KGF-2 protein using the baculovirus expression system (for review see: Summers, M.D. and Smith, G.E. 1987, A manual of methods for baculovirus vectors and insect cell culture procedures, Texas Agricultural Experimental Station Bulletin No. 1555). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhydrosis virus (AcMNPV) followed by the recognition sites for the restriction endonucleases BamHI and Asp718. The polyadenylation site of the simian virus (SV)40 is used for efficient polyadenylation. For an easy selection of recombinant viruses the beta-galactosidase gene from *E.coli* is inserted in the same orientation as the polyhedrin promoter followed by the polyadenylation signal of the polyhedrin gene. The polyhedrin sequences are flanked at both sides by viral sequences for the cell-mediated homologous recombination of co-transfected wild-type viral DNA. Many other baculovirus vectors could be used in place of pRG1 such as pAc373, pVL941 and pAcIM1 (Luckow, V.A. and Summers, M.D., *Virology*, 170:31-39).

The plasmid is digested with the restriction enzymes BamHI and Asp718. The DNA is then isolated from a 1% agarose gel using the commercially available kit (Qiagen, Inc., Chatsworth, CA). This vector DNA is designated V2.

Fragment F2 and the plasmid V2 are ligated with T4 DNA ligase. *E.coli* HB101 cells are then transformed and bacteria identified that contained the plasmid (pBackKGF-2) with the

KGF-2 gene using PCR with both cloning oligonucleotides. The sequence of the cloned fragment is confirmed by DNA sequencing.

5 μ g of the plasmid pBackKGF-2 is co-transfected with 1.0 μ g of a commercially available linearized baculovirus ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.) using the lipofection method (Felgner et al. Proc. Natl. Acad. Sci. USA, 84:7413-7417 (1987)).

1 μ g of BaculoGold™ virus DNA and 5 μ g of the plasmid pBackKGF-2 are mixed in a sterile well of a microtiter plate containing 50 μ l of serum free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards 10 μ l Lipofectin plus 90 μ l Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to the Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution is removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay performed similar as described by Summers and Smith (supra). As a modification an agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used which allows an easy isolation of blue stained plaques. (A detailed description of a "plaque assay" can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10).

Four days after the serial dilution, the viruses are added to the cells and blue stained plaques are picked with the tip of an Eppendorf pipette. The agar containing the

recombinant viruses is then resuspended in an Eppendorf tube containing 200 μ l of Grace's medium. The agar is removed by a brief centrifugation and the supernatant containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then stored at 4°C.

Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-KGF-2 at a multiplicity of infection (MOI) of 2. Six hours later the medium is removed and replaced with SF900 II medium minus methionine and cysteine (Life Technologies Inc., Gaithersburg). 42 hours later 5 μ Ci of 35 S-methionine and 5 μ Ci 35 S cysteine (Amersham) are added. The cells are further incubated for 16 hours before they are harvested by centrifugation and the labelled proteins visualized by SDS-PAGE and autoradiography.

Example 4

Expression of Recombinant KGF-2 in COS cells

The expression of plasmid, KGF-2 HA is derived from a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA fragment encoding the entire KGF-2 precursor and a HA tag fused in frame to its 3' end is cloned into the polylinker region of the vector, therefore, the recombinant protein expression is directed under the CMV promoter. The HA tag correspond to an epitope derived from the influenza hemagglutinin protein as previously described (I. Wilson, H. Niman, R. Heighten, A. Cherenson, M. Connolly, and R. Lerner, 1984, Cell 37:767, (1984)). The infusion of HA tag to the target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follows:

The DNA sequence encoding KGF-2, ATCC # 75977, is constructed by PCR using two primers: the 5' primer 5' CCCAAGCTTATGTGGAAATGGATACTGACACATTGTGCC 3' (SEQ ID No. 9) contains a Hind III site followed by 30 nucleotides of KGF-2 coding sequence starting from the initiation codon; the 3' sequence 5' TGCTCTAGACTAAGCGTAGTCTGGGACGTCGTATGGGTATGAGTG TACCACCATTGGAAGAAAGTGAGG 3' (SEQ ID No. 10) contains complementary sequences to an XbaI site, translation stop codon, HA tag and the last 32 nucleotides of the KGF-2 coding sequence (not including the stop codon). Therefore, the PCR product contains a Hind III site, KGF-2 coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and an XbaI site. The PCR amplified DNA fragment and the vector, pcDNA1/Amp, are digested with Hind III and Xba I restriction enzyme and ligated. The ligation mixture is transformed into E. coli strain XL1 Blue (Stratagene Cloning Systems, La Jolla, CA) the transformed culture is plated on ampicillin media plates and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by PCR and restriction analysis for the presence of the correct fragment. For expression of the recombinant KGF-2, COS cells are transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the KGF-2 HA protein is detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells are labelled for 8 hours with ³⁵S-cysteine two days post transfection. Culture media are then collected and cells are lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. et al., Id. 37:767 (1984)).

Both cell lysate and culture media are precipitated with a HA specific monoclonal antibody. Proteins precipitated are analyzed on 15% SDS-PAGE gels.

Example 5

Transcription and translation of recombinant KGF-2 in vitro:

A PCR product is derived from the cloned cDNA in the pA2 vector used for insect cell expression of KGF-2. The primers used for this PCR were:

5' ATTAACCCCTCACTAAAGGGAGGCCATGTGGAAATGGATACTGACACATTGTGCC 3'
(SEQ ID No. 11) and 5' CCCAAGCTTCCACAAACGTTGCCTTCCTCTATGAG 3'
(SEQ ID No. 12).

The first primer contains the sequence of a T3 promoter 5' to the ATG initiation codon. The second primer is complimentary to the 3' end of the KGF-2 open reading frame, and encodes the reverse complement of a stop codon.

The resulting PCR product is purified using a commercially available kit from Qiagen. 0.5 µg of this DNA is used as a template for an in vitro transcription-translation reaction. The reaction is performed with a kit commercially available from Promega under the name of TNT. The assay is performed as described in the instructions for the kit, using radioactively labeled methionine as a substrate, with the exception that only 1/2 of the indicated volumes of reagents are used and that the reaction is allowed to proceed at 33°C for 1.5 hours.

Five µl of the reaction is electrophoretically separated on a denaturing 10 to 15% polyacrylamide gel. The gel is fixed for 30 minutes in a mixture of water:Methanol:Acetic acid at 6:3:1 volumes respectively. The gel is then dried under heat and vacuum and subsequently exposed to an X-ray film for 16 hours. The film is developed showing the presence of a radioactive protein band corresponding in size to the conceptually translated KGF-2, strongly suggesting

that the cloned cDNA for KGF-2 contains an open reading frame that codes for a protein of the expected size.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

SEQUENCE LISTING

- (1) GENERAL INFORMATION:
 - (i) APPLICANT: GRUBER, ET AL.
 - (ii) TITLE OF INVENTION: Keratinocyte Growth Factor-2
 - (iii) NUMBER OF SEQUENCES: 12
 - (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: CARELLA, BYRNE, BAIN, GILFILLAN,
CECCHI, STEWART & OLSTEIN
 - (B) STREET: 6 BECKER FARM ROAD
 - (C) CITY: ROSELAND
 - (D) STATE: NEW JERSEY
 - (E) COUNTRY: USA
 - (F) ZIP: 07068
 - (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: 3.5 INCH DISKETTE
 - (B) COMPUTER: IBM PS/2
 - (C) OPERATING SYSTEM: MS-DOS
 - (D) SOFTWARE: WORD PERFECT 5.1
 - (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE: Concurrently
 - (C) CLASSIFICATION:
 - (vii) PRIOR APPLICATION DATA
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: FERRARO, GREGORY D.
- (B) REGISTRATION NUMBER: 36,134
- (C) REFERENCE/DOCKET NUMBER: 325800-261

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 201-994-1700
- (B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 627 BASE PAIRS
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

ATGTGGAAAT GGATACTGAC ACATTGTGCC TCAGCCTTTC CCCACCTGCC CGGCTGCTGC   60
TGCTGCTGCT TTTTGTTGCT GTTCTTGGTG TCTTCCGTCC CTGTCACCTG CCAAGCCCTT   120
GGTCAGGACA TGGTGTCAAC AGAGGCCACC AACTCTTCTT CCTCCTCCTT CTCCTCTCCT   180
TCCAGCGCGG GAAGGCATGT GCGGAGCTAC AATCACCTTC AAGGAGATGT CCGCTGGAGA   240
AAGCTATTCT CTTTCACCAA GTACTTTCTC AAGATTGAGA AGAACGGGAA GGTCAGCGGG   300
ACCAAGAAGG AGAACTGCCC GTACAGCATC CTGGAGATAA CATCAGTAGA AATCGGAGTT   360
GTTGCCGTCA AAGCCATTAA CAGCAACTGT TACTTAGCCA TGAACAAGAA GGGGAAACTC   420
TATGGCTCAA AAGAATTTAA CAATGACTGT AAGCTGAAGG AGAGGATAGA GGAAAATGGA   480
TACAATACCT ATGCATCATT TAACTGGCAG CATAATGGGA GGCAAATGTA TGTGGCATTG   540
AATGGAAAAG GAGCTCCAAG GAGAGGACAG AAAACACGAA GGAAAAACAC CTCTGCTCAC   600
TTTCTTCCAA TGGTGGTACA CTCATAG                                     627

```


Asn Gly Lys Gly Ala Pro Arg Arg Gly Gln Lys Thr Arg Arg Lys		
145	150	155
Asn Thr Ser Ala His Phe Leu Pro Met Val Val His Ser		
160	165	170

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 36 BASE PAIRS
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCCCACATGT GGAAATGGAT ACTGACACAT TGTGCC

36

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 35 BASE PAIRS
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCCAAGCTTC CACAAACGTT GCCTTCCTCT ATGAG

35

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 36 BASE PAIRS

- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CATGCCATGG CGTGCCAAGC CCTTGGTCAG GACATG

36

- (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 35 BASE PAIRS
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: SINGLE
 - (D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCCAAGCTTC CACAAACGTT GCCTTCCTCT ATGAG

35

- (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 35 BASE PAIRS
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: SINGLE
 - (D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCGGGATCCG CCATCATGTG GAAATGGATA CTCAC

35

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 26 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGCGGTACCA CAAACGTTGC CTCCT

26

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 39 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CCCAAGCTTA TGTGGAAATG GATACTGACA CATTGTGCC

39

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 69 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGCTCTAGAC TAAGCGTAGT CTGGGACGTC GTATGGGTAT GAGTGTACCA CCATTGGAAG 60
AAAGTGAGG 69

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 54 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATTAACCCCTC ACTAAAGGGA GGCCATGTGG AAATGGATAC TGACACATTG TGCC 54

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 35 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCCAAGCTTC CACAAACGTT GCCTTCCTCT ATGAG 35

WHAT IS CLAIMED IS:

1. An isolated polynucleotide selected from the group consisting of:
 - (a) a polynucleotide encoding the polypeptide having the deduced amino acid sequence of SEQ ID No. 2 or a fragment, analog or derivative of said polypeptide;
 - (b) a polynucleotide encoding the polypeptide having the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 75977 or a fragment, analog or derivative of said polypeptide.
2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.
3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.
4. The polynucleotide of Claim 1 wherein the polynucleotide is genomic DNA.
5. The polynucleotide of Claim 2 wherein said polynucleotide encodes a polypeptide having the deduced amino acid sequence of SEQ ID No. 2.
6. The polynucleotide of Claim 2 wherein said polynucleotide encodes the polypeptide encoded by the cDNA of ATCC Deposit No. 75977.
7. The polynucleotide of Claim 1 having the coding sequence as shown in SEQ ID No. 1.
8. The polynucleotide of Claim 2 having the coding sequence of the polypeptide deposited as ATCC Deposit No. 75977.
9. A vector containing the DNA of Claim 2.
10. A host cell genetically engineered with the vector of Claim 9.
11. A process for producing a polypeptide comprising: expressing from the host cell of Claim 10 the polypeptide encoded by said DNA.

12. A process for producing cells capable of expressing a polypeptide comprising genetically engineering cells with the vector of Claim 9.

13. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having KGF-2 activity.

14. A polypeptide selected from the group consisting of (i) a polypeptide having the deduced amino acid sequence of SEQ ID No. 2 and fragments, analogs and derivatives thereof and (ii) a polypeptide encoded by the cDNA of ATCC Deposit No. 75977 and fragments, analogs and derivatives of said polypeptide.

15. The polypeptide of Claim 14 wherein the polypeptide is KGF-2 having the deduced amino acid sequence of SEQ ID No. 2.

16. An antibody against the polypeptide of claim 14.

17. A compound effective as an agonist to the polypeptide of claim 14.

18. A compound effective as an antagonist against the polypeptide of claim 14.

19. A method for the treatment of a patient having need of KGF-2 comprising: administering to the patient a therapeutically effective amount of the polypeptide of claim 14.

20. A method for the treatment of a patient having need to inhibit KGF-2 comprising: administering to the patient a therapeutically effective amount of the compound of Claim 18.

21. The method of Claim 19 wherein said therapeutically effective amount of the polypeptide is administered by providing to the patient DNA encoding said polypeptide and expressing said polypeptide in vivo.

22. A process for identifying compounds active as agonists to KGF-2 comprising:

(a) combining a compound to be screened, and a reaction mixture containing cells under conditions where

the cells are normally stimulated by KGF-2, said reaction mixture containing a label incorporated into the cells as they proliferate; and

(b) determining the extent of proliferation of the cells to identify if the compound is an effective agonist.

23. The process of claim 22 for identifying compounds active as antagonists to KGF-2, wherein KGF-2 is added to the combination of step (a).

24. A process for diagnosing a disease or a susceptibility to a disease related to an under-expression of the polypeptide of claim 14 comprising:

isolating a nucleic acid sequence encoding said polypeptide from a sample derived from a host; and

determining a mutation in the nucleic acid sequence encoding said polypeptide.

25. A diagnostic process comprising:

analyzing for the presence of the polypeptide of claim 14 in a sample derived from a host.

FIG. 1A

ATGTGGAATGGATACTGACACATTGTGCTCAGCCTTTTCCCCACCTGCCCGGCTGCTGC
-----+-----+-----+-----+-----+-----+-----+-----+
TACACCTTTACCTATGACTGTGTAAACGGAGTCGGAAGGGTGGACGGGCCGACGACG
60 -

M W K W I L L T H C A S A F P H L L P G C C

61
TGCTGCTGCTTTTGTGTGCTGTTCTTGGTGCTTCCGTCCCTGTCACTGCCAAGCCCTT
-----+-----+-----+-----+-----+-----+
ACGACGACGAAACACGACAAGAACACAGAACGACAGGACAGTGCACGGTTCGGGAA
120

C C C F L L L F L V S S V P V T C Q A L

121
GGTCAGGACATGGTGTCAACAGAGGCCACCAACTCTTCTTCCTCCTCCTCTCCTCTCTCT
-----+-----+-----+-----+-----+-----+-----+-----+
CCAGTCCCTGTACCAACAGTGTCTCCGGTGGTTGAGAAAGAGGAGGAAGAGGAGAGGGA
180

C
Q
Q
D
M
V
S
P
E
A
T
N
S
S
S
S
S
L
S
S
I

18) TCCAGCGCGGGAAGGCATGT9CGGAGCTACAATCACCTTCAAGGAGATGTCCGCTGGAGA
 -----+-----+-----+-----+-----+-----+-----+
 AGTCCGCGCCCTTCCGTACACGCCCTCGATGTTAGTGGAAAGTTCTCTACAGCGGACCTCT 240

S S A C R H V R S Y N H L Q C D V R W R -

MATCH WITH FIG. 1B

MATCH WITH FIG. 1A FIG. 1B

241 AAGCTATTCTCTTTCACCAAGTACTTTCTCAAGATGAGAAACGGGAAGTCAGCGGG
 -----+-----+-----+-----+-----+-----+-----+
 TTCGATAAGAGAAAGTGGTTCATGAAGAGTTCTAACTCTTCTTGCCCTTCCAGTCGCCC 300

K L F S F T K Y F L K I E K N G K V S G -
 ACCAAGAGGAGAACTGCCCGTACAGCATCCTGGAGATAACATCAGTAGAAATCGGAGTT
 -----+-----+-----+-----+-----+-----+-----+ 360
 TGGTTCTTCCTCTTGACGGGCATGTCGTAGGACCTCTATTGTAGTCATCTTTAGCCTCAA

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 T K K E N C P Y S I L E I T S V E I G V -
 GTTCCCGTCAAAGCCATTAAACAGCAACTATTACTTAGCCATGAACAAGGGAACCTC
 -----+-----+-----+-----+-----+-----+-----+ 420
 CAACGGCAGTTTCGGTAATTGTCGTTGATAATGAATCGGTACTGTCTTCCCCCTTTGAG

V A V K A I N S N Y Y L A M N K K G K L -
 TATGGCTCAAAGAAATTTAACAATGACTGTAAAGCTGAAGGAGAGGATAGAGGAAATCGA
 -----+-----+-----+-----+-----+-----+-----+ 480
 ATACCGAGTTTCTTAAATTGTTACTGACATTGCACTTCCTCTCCTATCTCCTTTTACCT
 Y G S K E F N N D C K L K E R I E E N G -

MATCH WITH FIG. 1C

MATCH WITH FIG. 1B FIG. 1C

481 TACAATACCTATGCATCATTTAACTGGCAGCATAATGGAGGCAAAATGTATGTGGCATTG
 -----+-----+-----+-----+-----+-----+-----+
 ATGTTATGGATACGTAAGTAATTGACCGTCGTATTACCCCTCCGTTTACATACACCGTAAC
 540
 Y N T Y A S F N W Q H N G R Q M Y V A L -
 AATGGAAGGAGCTCCAAGGAGAGGACAGAGAAACACGAGAAACACCTCTGCTCAC
 -----+-----+-----+-----+-----+-----+-----+
 TTACCTTTTCCCTCGAGGTTCCCTCTCCTGCTCTTTTGTGCTTCTTCTTGTGAGACGAGTG
 541
 N G K G A P R R G Q K T R R K N T S A H -
 TTTCTTCCAATGGTGTAACACTCATAG
 -----+-----+-----+-----+-----+-----+-----+
 AAAGAAGGTTACCACCATGTGAGTATC
 601
 F L P M V V H S *
 627

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FIG. 2A

FGF4	1	MS.GPGTAAV	ALLPAVLLAL	LA.....	.PWAGRGGAA	APTAPNGTLE	50
FGF6		MSRGAGRLQG	TLWALVFLGI	LV.....	.GMVVPSPAG	TR.ANNTLLD	
FGF5	MSL	SFLLLLFFSH	LILSAWAHGE	KRLAPKGQPG	PAATDRNPIC	
FGF1		
FGF2		
FGF9		
FGF7		
KGF2	 MHKW	ILTWILPTLLYRSCF	HIICLVGTIS	
FGF3	 MWKW	ILTHCASAFF	HLPGCCCCCF	LLFLVSSVP	
FGF8	MGL	IWLLLSLLE	
		MGSPRSALSC	LLLHLLVLCL	QAQVRSAAQK	RGPAGNPAD	TLGQGHEDRP	

FGF4	51	AELERRWESL	VALSLARLPV	AA..QPKEAA	VQSGAGDY..	...LLGIKRL	100
FGF6		S...RGWGTL	LSRSRAGLAG	EI.....AG	VNWESG.Y..	...LVGIKRQ	
FGF5		SSSRQSSSA	MSSSSASSSP	AASLGSQSG	LEQSSFQW..	...SPSGRRT	
FGF1	MAEG	EITTFALTTE	KFN...LPPGN..	...YK...KP	
FGF2	MAAG	SITTLPALPE	DGSGAFPPGH..	...FK...DP	
FGF9		FGNVPVLPVD	SPVLLSDHLG	QSEAGGLPRG	PAVTDLDH..	...LKGILRR	
FGF7		LACNDMTPEQ	M...ATNVNCSSPE	RHTRSVDY..	...MEGGDIR	
KGF2		VTCQALGQDM	VSPEATNSSS	SSFSSPSSAG	RHVRSYNH..	...LQ.GDVR	
FGF3		PGWPAAGPGARLRRDAG	GRGGVYEH..	...L.GGAPR	
FGF8		FGQRSRAGKN	FTNPAPNYPE	EGSKEQRDSV	LPKVTQRHVR	EQSLVTDQLS	

MATCH WITH FIG. 2B

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MATCH WITH FIG. 2A

FIG. 2B

101

FGF4	RRL.....YC	NVGIGFHLQA	LPDGRIGGAH	ADT.RDSLLE	LSPVERGV.V
FGF6	RRL.....YC	NVGIGFHLQV	LPDGRISGTH	EEN.PYSLLE	ISTVERGV.V
FGF5	GSL.....YC	RVGIGFHLQI	YPDGKVNESH	EAN.MLSVLE	IFAVSQGI.V
FGF1	KLL.....YC	SNG.GHFLRI	LPDGTVDGTR	DRSDQHIQLQ	LSAESVGE.V
FGF2	KRL.....YC	KNG.GFFLRI	HPDGRVDGVR	EKSDPHIKLQ	LQAEERGV.V
FGF9	RQL.....YC	R.T.GFHLRI	FPNGTIQGTR	KDHSRFGILE	FISIAVGL.V
FGF7	VRR.....LF	CRT.QWYLR	DKRGKVKGTQ	EMKNYNYNME	IRTVAVGI.V
KGF2	WRK.....LF	SFT.KYFLKI	EKNGKVSGTK	KENCOPYSILE	ITSVEIGV.V
FGF3	RRK.....LY	CAT.KYHLQL	HPSGRVNGSL	.ENSAYSILE	ITAVEVGI.V
FGF8	RRLIRTYQLY	SRTSGKHVQV	LANKRINAMA	EDGDPFAKLI	VETDTFGSRV

150

151

FGF4	SIFGVASRFF	VAMSSKGRLY	G.SPFFTDEC	TFRKILLPNN	YNAYESYKYP
FGF6	SLFGVRSALF	VAMNSKGRLY	A.TPSFQEEC	KFRRTLLPNN	YNAYESDLYQ
FGF5	GIRGVFSNKF	LAMSKKGRLLH	A.SAKFTDDC	KFRERFOENS	YNTYASAIHR
FGF1	YIKSTETGQY	LAMDTDGLLY	G.SQTPNEEC	LFLERLEENH	YNTYISKKH.
FGF2	SIKGVCANRY	LAMKEDGRLL	A.SKCVTDEC	FFFERLESNN	YNTYRSRKY.
FGF9	SIRGVDSGLY	LGMNEKGELY	G.SEKLTQEC	VFERQFEENW	YNTYSSNLKY
FGF7	AIKGVSESEFY	LAMNKEGKLY	A.KKECNEDC	NFKELILENH	YNTYAS....
KGF2	AVKAINSNYY	LAMNKGKRLY	G.SKEFNNDK	KLKERIEENG	YNTYAS....
FGF3	AIRGLFSGRY	LAMNKRGRLY	A.SEHYSAEC	EFVERIHELG	YNTYASRLYR
FGF8	RVRGAETGLY	ICMNRKGRLL	AKSNGKGKDC	VFTIIVLENN	YTALQNAKY.

200

MATCH WITH FIG. 2C

FIG. 2C

MATCH WITH FIG. 2B

201

FGF4	GM.....	FI	ALSKNGKTKK	G..	NRVSPTM	250	KVTHFLPRL.
FGF6	GT.....	YI	ALSKYGRVKR	G..	SKVSPIM		TVTHFLPRI.
FGF5	TEKTGREWYV		ALNKRKGAKR	GCS	PRVKPQH		ISTHFLPRFK
FGF1AEKNWFV		GLKKNGSCKR	G..	PRTHYGQ		KAILFLPLPV
FGF2T...SWYV		ALKRTGQYKL	G..	SKTGPGQ		KAILFLPMSA
FGF9DTGRRYV		ALNKDGTPRE	G..	TRTKRHQ		KFTHFLPRPV
FGF7	AKW	THNGGEM.FV	ALNQKGIPVR	G..	KTKKEQ		KTAHFLPMAI
KGF2	FNW	QHNGRQM.YV	ALNGKGAPRR	G..	QKTRRKN		TSAHFLPMVV
FGF3	TVSSTPGARR	QPSAERLWYV	SVNGKGRPRR	G..	EKTRRTQ		KSSLFLPRVL
FGF8	EGWYM	AFTRKGRPRK	G..	SKTRQHQ		REVHFMKRLP

251

FGF4	300
FGF6
FGF5	QSEQPELSFT	VTVPEKKNPP
FGF1	SSD.....	SPIKSKIPLS	APRKN	TNSVK		YRLKFRFG..
FGF2	KS.....
FGF9	DPDKVPELYK	DILSQS...
FGF7	T.....
KGF2	HS.....
FGF3	DHRDHEMVRQ	LQSGLP RP PG
FGF8	RGHHTTEQSL	RFEFLNYP PF	TRSLRGSQRT	WAVEPR...

MATCH WITH FIG. 2D

FIG. 2D

MATCH WITH FIG. 2C

EGF4	301
EGF6	
EGF5	
EGF1	
EGF2	
EGF9	
EGF7	
KGF2	
EGF3		LEASAH
EGF8	

INTERNATIONAL SEARCH REPORT

 International application No.
 PCT/US95/01790

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 21/00; A61K 38/18; C07K 14/50

US CL : 530/399; 536/23.5; 435/69.4; 320.1; 252.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/399; 536/23.5; 435/69.4; 320.1; 252.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

 APS, dialog, GenBank
 search terms: KGF-2

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	Science, Volume 245, issued 18 August 1989, P. W. Finch et al., "Human KGF Is FGF-Related with Properties of a Paracrine Effector of Epithelial Cell Growth," pages 752-755, especially figure 1.	1-4, 9-14, 19 ----- 5-8, 15
X ---- Y	Proceedings of the National Academy of Sciences USA, Volume 89, issued October 1992, M J. Kelley et al., "Emergence of the keratinocyte growth factor multigene family during the great ape radiation," pages 9287-9291, especially figure 3.	1-4, 9-14, 19 ----- 5-8, 15
X	In Vitro Cellular Developmental Biology, Volume 27A, issued June 1991, G. Yan et al, "Sequence of Rat Keratinocyte Growth Factor (Heparin-Binding Growth Factor Type 7)," pages 437-438, especially figure 2.	1-4, 9-14, 19

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

26 MAY 1995

Date of mailing of the international search report

07 JUN 1995

 Name and mailing address of the ISA/US
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01790

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- A	Mechanisms of Development, Volume 45, issued 1994, I. J. Mason et al., "FGF-7 (keratinocyte growth factor) expression during mouse development suggests roles in myogenesis, forebrain regionalisation and epithelial-mesenchymal interactions," pages 15-30, especially figure 1.	1-4, 9-14, 19 ----- 20,21
X	Molecular and Cellular Biology, Volume 13, number 7, issued July 1993, M. Miyamoto et al., "Molecular Cloning of a Novel Cytokine cDNA Encoding the Ninth Member of the Fibroblast Growth Factor Family, Which Has a Unique Secretion Property," pages 4251-4259, especially figure 1.	1-4, 9-14, 19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01790**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-15, 19-21
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-13, drawn to the DNA encoding KGF-2, a vector, a host cell, a process for producing a polypeptide, a process for producing cells, and an isolated DNA.

Group II, claims 14-15, 19-21, drawn to KGF-2 protein and a method of use.

Group III, claims 16-18, 22-23, drawn to an antibody to KGF-2.

Group IV, claims 24-25, drawn to a method of diagnosing a disease comprising mutations in the KGF-2 DNA sequence.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I-III lack the same or corresponding special technical features because the DNA of I, the protein of II and the antibody of III are materially distinct compounds having structural and functional properties that distinguish them, each from the other. KGF-2 does not define a special technical feature defining over the prior art because KGF-2 was known in the prior art by isolation from natural sources. The methods of Groups I and IV lack the same or corresponding technical because they are directed to materially distinct processes distinguished by their materially different process steps and purposes. Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

FIG. 1A

1 ATGTGGAATGGATACTGACACATTTGTCCTCAGCCTTTCCCCACCTGCCCGGCTGCTGC

 TACACCTTTACCTATGACTGTGTACACGGAGTCGGAAGGGTGGACGGCGGACGACG
 60
 M W K W I L L T H C A S A F P H L P G C C
 TGCTGCTGCTTTTGTGCTGTCTTGGTGTCTTCCGTCCTGTCACTGCCAAGCCCTT

 ACGACGAGAAACAACGACACAGAACCCACAGAACGACAGTGGACGGTTTCGGGAA
 120
 C C C F L L L L F L V S S V P V T C Q A L
 GGTGAGGACATGGTGTACCCAGAGGCCACCACTCTTCTTCCTCCTCTCTCTCTCTCT

 CCAGTCTGTACACAGTGGTCTCCCGTGGTTGAGAGAGGAGGAGGAGAGAGAGGA
 180
 G Q D M V S P E A T N S S S S S F S S P
 TCCAGCGCGGAGGCAATGTGCGAGCTACAAATCACCTTCAAGGAGATGTCCGCTGGAGA

 AGGTGCGGCCCTTCGGTACACGCCCTCGATGTGTAGTGGAGTTCTCTACAGCGACCTCT
 240
 S S A G R H V R S Y N H L Q G D V R W R
 MATCH WITH FIG. 1B

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FIG. 2A

FGF4	1	MS.GPGTAAV	ALLPAVLLAL	LA.....	.PWAGRGAA	APTAPNGTLE	50
FGF6		MSRGAGRLQG	TLWALVFLGI	LV.....	.GMVVPSPAG	TR.ANNTLLD	
FGF5	MSL	SFLLLFFSH	LILSAWAHGE	KRLAPKGQPG	PAAATDRNPIG	
FGF1		
FGF2		
FGF9		
FGF7		
KGF2		
FGF3		
FGF8		MGSPRSALSC	LLLHLVLVLC	QAQVRSAAQK	RGPGAGNPAD	TLGQGHEDRP	
FGF4	51	AELERRWESL	VALSLARLPV	AA..QPKEAA	VQSGAGDY..	...LLGIKRL	100
FGF6		S...RGWGTL	LSRSRAGLAG	EI.....AG	VNWESG.Y..	...LVGIKRQ	
FGF5		SSSRQSSSA	MSSSSASSP	AASLGSGSG	LEQSSFQW..	...SPSGRRT	
FGF1	MAEG	EITTFALT	KFN...LPPGN..	...YK...KP	
FGF2	MAAG	SITTLPALPE	DGSGAFPPGH..	...FK...DP	
FGF9		FGNVPVLPVD	SPVLLSDHLG	QSEAGGLPRG	PAVTDLDH..	...LKGILRR	
FGF7		LACNDMTPEQ	M...ATNVNCSSPE	RHTRSYDY..	...MEGGDIR	
KGF2		VTCQALGQDM	VSPEATNSSS	SSFSFSSSAG	RHVRSYNH..	...LQ.GDVR	
FGF3		PGWPAAGPGARLRRDAG	GRGGVYEH..	...L.GGAPR	
FGF8		FGQRSRAGKN	FTNPAPNYPE	EGSKEQRDSV	LPKVTQRHVR	EQSLVTDQLS	

MATCH WITH FIG. 2B

MATCH WITH FIG. 2A

101

FGF4	RRL.....YC	NVGIGFHLQA	LPDGRIGGAH	ADT.RDSSLLE	LSPVERGV.V
FGF6	RRL.....YC	NVGIGFHLQV	LPDGRISGTH	EEN.PYSLLE	ISTVERGV.V
FGF5	GSL.....YC	RVGIGFHLQI	YPDGKVNQSH	EAN.MLSVLE	IFAVSQGI.V
FGF1	KLL.....YC	SNG.GHFLRI	LPDGTVDGTR	DRSDQHIQLQ	LSAESVGE.V
FGF2	KRL.....YC	KNG.GFFLRI	HPDGRVDGVR	EKSDPHIKLQ	LQAEERGV.V
FGF9	RQL.....YC	R.T.GFHLRI	FPNGTIQGTR	KDHSRFGILE	FISIAVGL.V
FGF7	VRR.....LF	CRT.QWYLRI	DKRGKVKGTQ	EMKNNYNIME	IRTVAVGI.V
KGF2	WRK.....LF	SFT.KYFLKI	EKNQKVSQTK	KENCPSYLE	ITSVIEIGV.V
FGF3	RRK.....LY	CAT.KYHLQL	HPSGRVNGSL	.ENSAYSILE	ITAVEVGI.V
FGF8	RRLIRTYQLY	SRTSGKHVQV	LANKRINAMA	EDGDPFAKLI	VETDTFGSRV

150

151

FGF4	SIFGVASRFF	VAMSSKGLY	G.SPFFTDEC	TFKEILLPNN	YNAYESYKYP
FGF6	SLFGVRSALF	VAMNSKGRLY	A.TPSFQEEC	KFRBTLLPNN	YNAYESDLYQ
FGF5	GIRGVFSNKF	LAMSKKGLH	A.SAKFTDDC	KFRERFQENS	YNTYASAIHR
FGF1	YIKSTETGQY	LAMDTDGLLY	G.SQTPNEEC	LFLERLEENH	YNTYISKKH.
FGF2	SIRGVCANRY	LAMKEDGRLL	A.SKCVTDEC	FFFERLESNN	YNTYRSRKY.
FGF9	SIRGVDSGLY	LGMNEKGELY	G.SEKLTQEC	VFERBQFEENW	YNTYSSNLYK
FGF7	AIKGVSESEFY	LAMNKEGKLY	A.KKBCNEDC	NFKELILENH	YNTYAS....
KGF2	AVKAINSNYY	LAMNKGKGLY	G.SKEFNNDG	KLKERIEENG	YNTYAS....
FGF3	AIRGLFSGRY	LAMNKRGRLY	A.SEHYSAEC	EFVERIHBLG	YNTYASRLYR
FGF8	RVRGAETGLY	ICMNXKGLI	AKSNGKGRDC	VFTIIVLENN	YATALQNAKY.

200

MATCH WITH FIG. 2C

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FIG. 2C

MATCH WITH FIG. 2B

201

EGF4	GM.....	FI	ALSKNGKTKK	G..	NRVSPTM	250	KVTHFLPRL.
EGF6	GT.....	YI	ALSKYGRVKK	G..	SKVSPIM		TVTHFLPRI.
EGF5	TEKTGREWYV		ALNKRKGAKR	GCS	PRVKPQH		ISTHFLPRFK
EGF1	AEKNWFV	GLKKNGSCKR	G..	PRTHYGQ		KAILFLPLPV
EGF2	T...SWYV	ALKRTGQYKL	G..	SKTGPGQ		KAILFLPMSA
EGF9	HV.....	DTGRRYYV	ALNKDGTPRE	G..	TRTKRHQ		KFTHFLPRPV
EGF7	AKW	THNGGEM.FV	ALNQKGIPVR	G..	KKTKKEQ		KTAHFLPMAI
KGF2	FNW	QHNGRQM.YV	ALNGKGAPRR	G..	QKTRRKN		TSAHFLPMVY
EGF3	TVSSTPGARR	QPSAERLWYV		SVNGKGRPRR	G..	FKTRRTQ		KSSLFLPRVL
EGF8	EGWYM	AFTRKGRPRK	G..	SKTRQHQ		REVHFMKRLP

251

EGF4	300
EGF6
EGF5	QSEQPELSFT	VTVPEKKNPP		SPIKSKIPLS	APRKN	TNSVK		YRLKFRFG..
EGF1	SSD.....
EGF2	KS.....
EGF9	DPDKVPELYK	DILSQS.....
EGF7	T.....
KGF2	HS.....
EGF3	DHRDHENVRO	LQSGLEPRPPG		KGVPERRRQ	KQSPDN	LEPS		HVQASRLGSQ
EGF8	RGHHTTEQSL	RFEFLNYPPE		TRSLRGSQRT	WAPEPR

MATCH WITH FIG. 2D

FIG. 2D

MATCH WITH FIG. 2C

EGF4	301
EGF6	
EGF5	
EGF1	
EGF2	
EGF9	
EGF7	
KGF2	
EGF3		LEASAH
EGF8	

